

Step 1: Reverse transcription--cDNA synthesis from reverse primer mix.

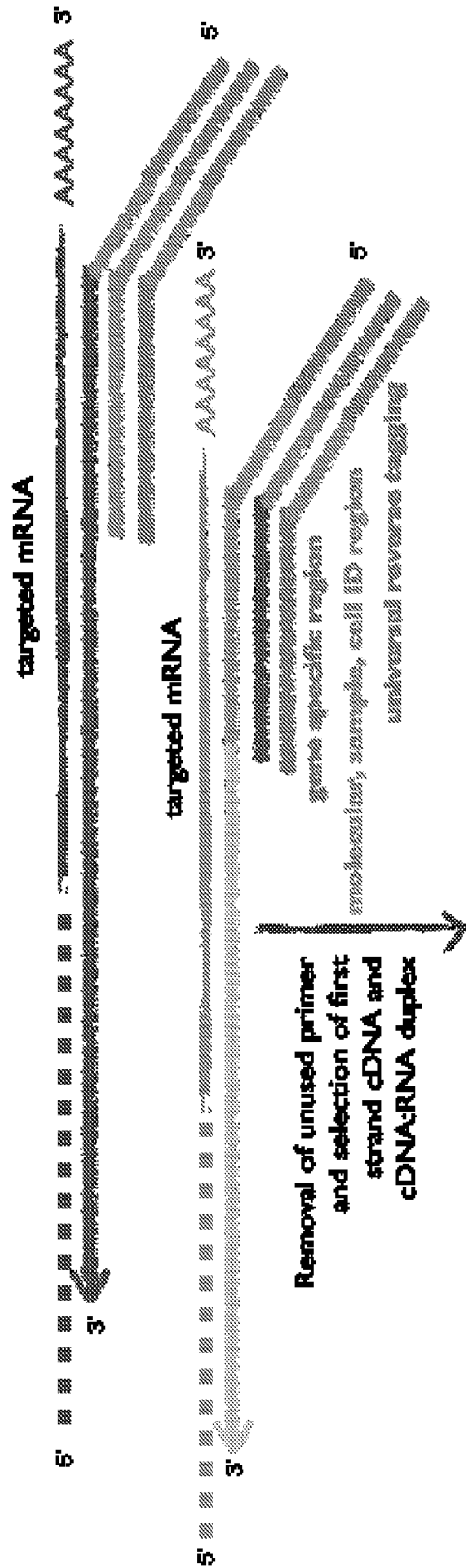


FIG. 1A

Step 2: One cycle second strand DNA synthesis from the forward primer mix.

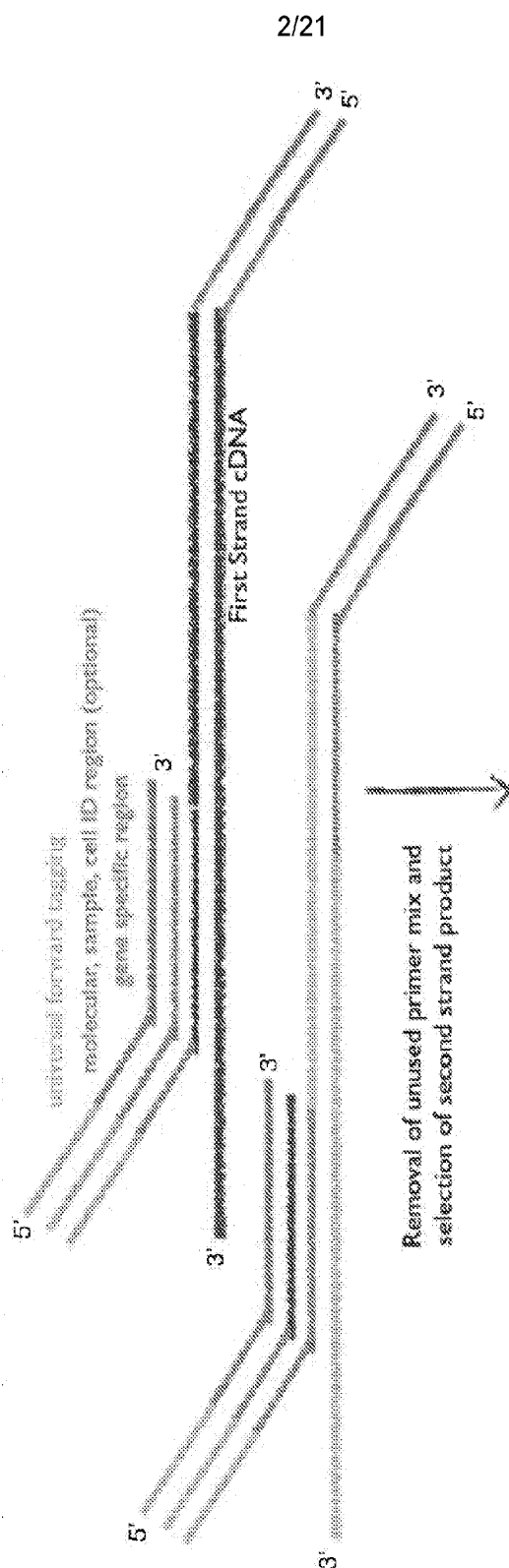
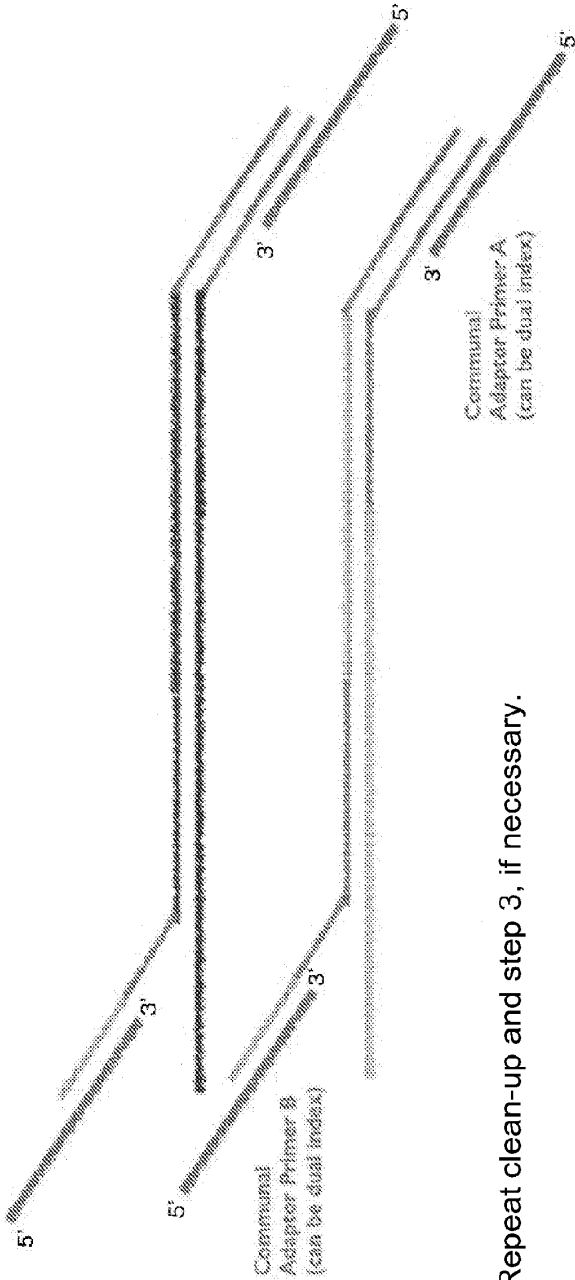


FIG. 1B

Step 3: Two-step PCR to complete the sequencing adaptor and amplify the tagged products from the first and second cycles.



Step 4: Repeat clean-up and step 3, if necessary.

FIG. 1C

Step 1: A first primer mix is used in one cycle tagging with gDNA. The first primer mix can be forward primers only, reverse primers only, or a mixture of forward and reverse primers used for strategic tagging.

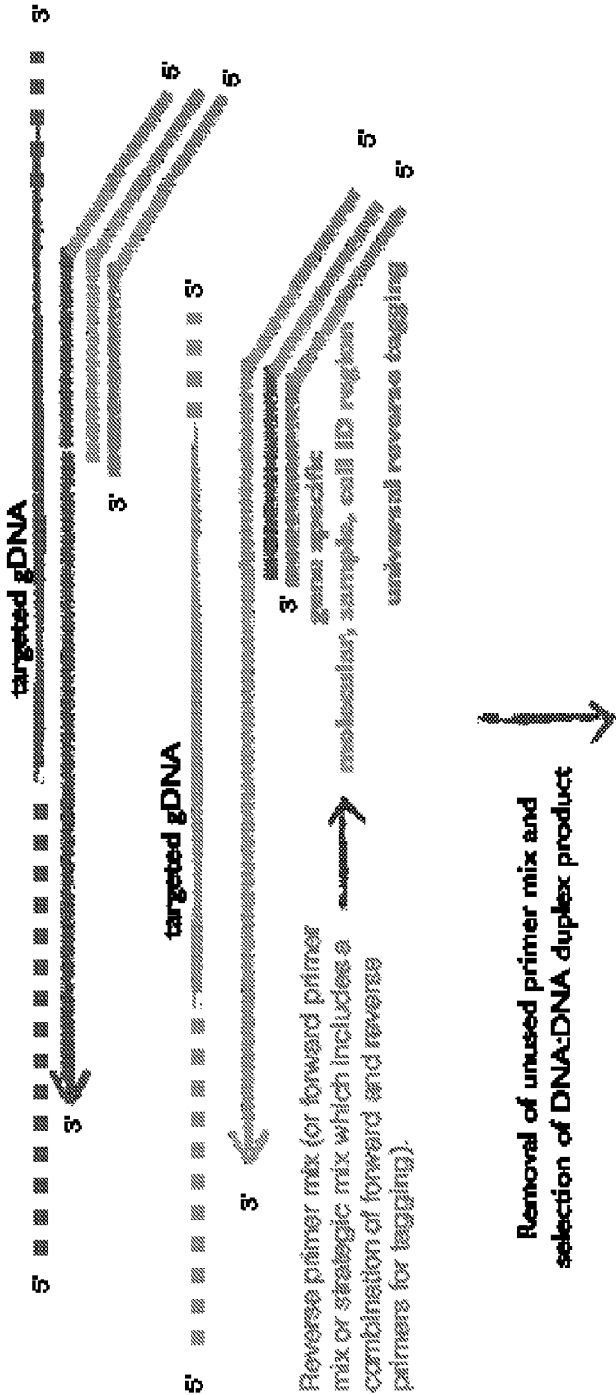


FIG. 2A

Step 2: One cycle second strand DNA synthesis with a second primer mix. The second primer mix can be forward primers only, reverse primers only, or a mixture of forward and reverse primers used for strategic tagging. The selection of the primers to include in the mix depends on what was used in the first cycle.

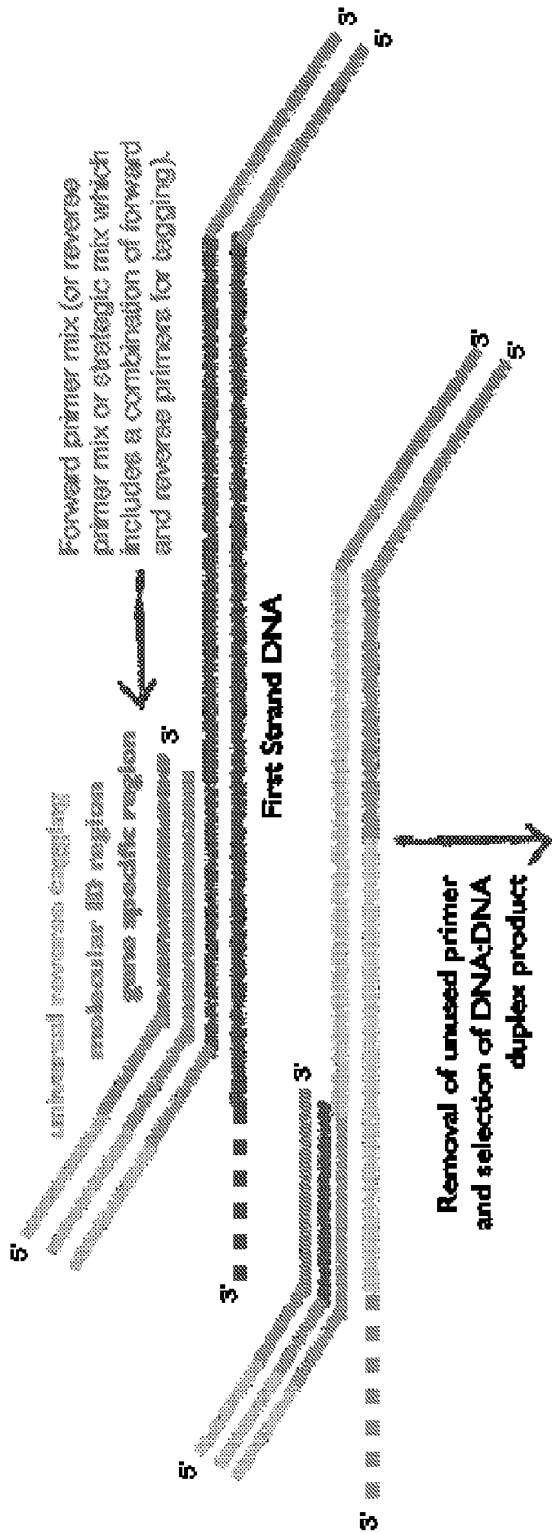
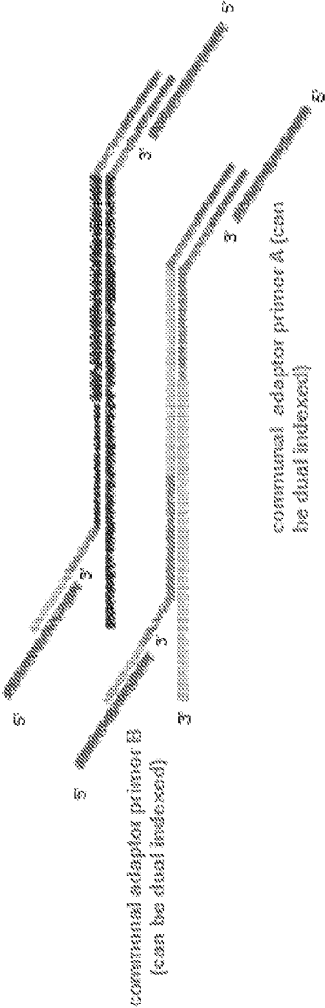


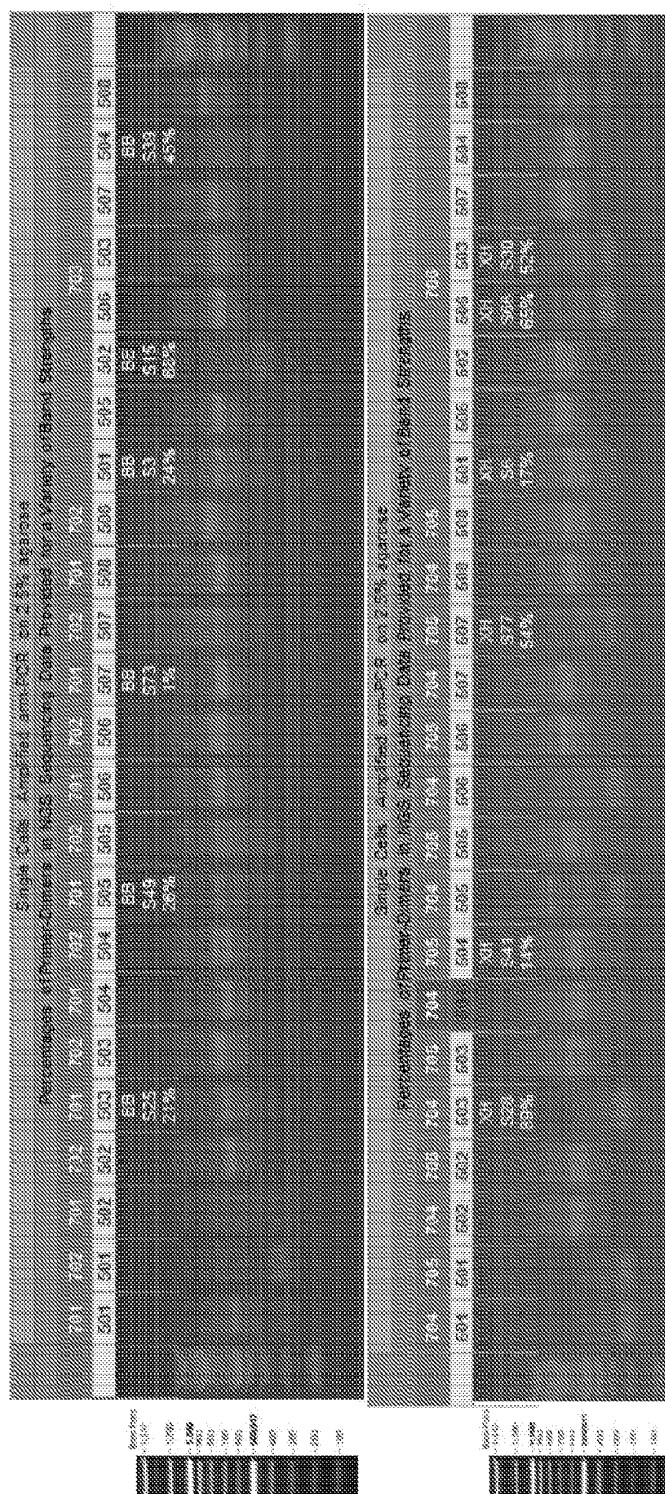
FIG.2B

Step 3: Two-step PCR to complete the sequencing adaptor and amplify the tagged products from the first and second cycles.



Step 4: Repeat clean-up and step 3 if necessary.

FIG. 2C



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Primer Combination	Reverse Primer (R1)	Forward primer (R2)	Frequency	% in Trash	% for Total Reads	Sum % in Trash	Totals reads in 12 samples reads as trash % trash
FOXP3-RI-09 + ITRAV_2	FOXP3-RI-09	ITRAV_2	12	10.5%	3.2%	10.5%	873862
BCL6RI_MD_11 + ITRAV_40	BCL6RI_MD_11	ITRAV_40	12	10.0%	3.1%	20.5%	270898
ITRAC + ITRAV_2	ITRAC	ITRAV_2	12	6.1%	1.9%	26.6%	31%
IL-17F-RI-09 + ITRAV_41	IL-17F-RI-09	ITRAV_41	12	5.5%	1.7%	32.1%	
BCL6RI_MD_11 + ITRAV_23	BCL6RI_MD_11	ITRAV_23	12	4.6%	1.4%	36.7%	
IL-17F-RI-09 + ITRAV_2	IL-17F-RI-09	ITRAV_2	12	2.6%	0.8%	39.2%	
CTLA-4-RI-09 + CD8-FI	CTLA-4-RI-09	CD8-FI	10	3.1%	1.0%	42.3%	
IL-17F-RI-09 + ITRAV_34	IL-17F-RI-09	ITRAV_34	10	2.2%	0.7%	44.5%	
Eomes-RI-09 + ITRAV_2	Eomes-RI-09	ITRAV_2	7	2.1%	0.6%	46.6%	
Eomes-RI-09 + ITRAV_42	Eomes-RI-09	ITRAV_42	5	2.1%	0.7%	48.7%	
ITRBC + RUNX3FI_MD	ITRBC	RUNX3FI_MD	3	2.1%	0.7%	50.8%	
IL-17F-RI-09 + ITRAV_23	IL-17F-RI-09	ITRAV_23	3	1.7%	0.5%	52.5%	
FOXP3-RI-09 + IL-22-FI	FOXP3-RI-09	IL-22-FI	3	1.6%	0.5%	54.1%	
FOXP3-RI-09 + CD8-FI	FOXP3-RI-09	CD8-FI	2	4.3%	1.3%	58.5%	
Eomes-RI-09 + ITRBV_15	Eomes-RI-09	ITRBV_15	2	1.8%	0.6%	60.3%	
TNFA-RI-09 + ITRAV_42	TNFA-RI-09	ITRAV_42	1	1.8%	0.6%	62.1%	
ITRAC + ITRAV_34	ITRAC	ITRAV_34	1	1.0%	0.4%	63.5%	

FIG. 3B

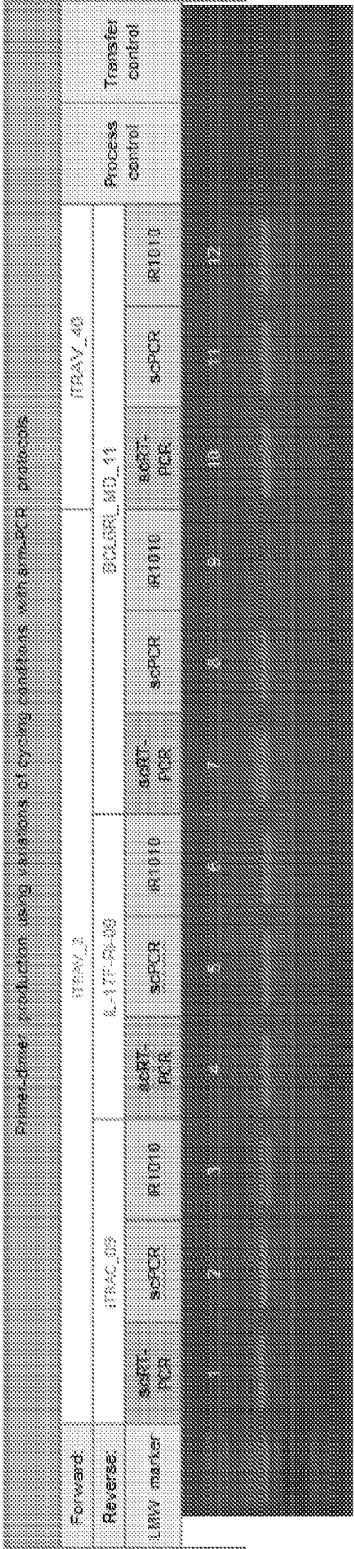
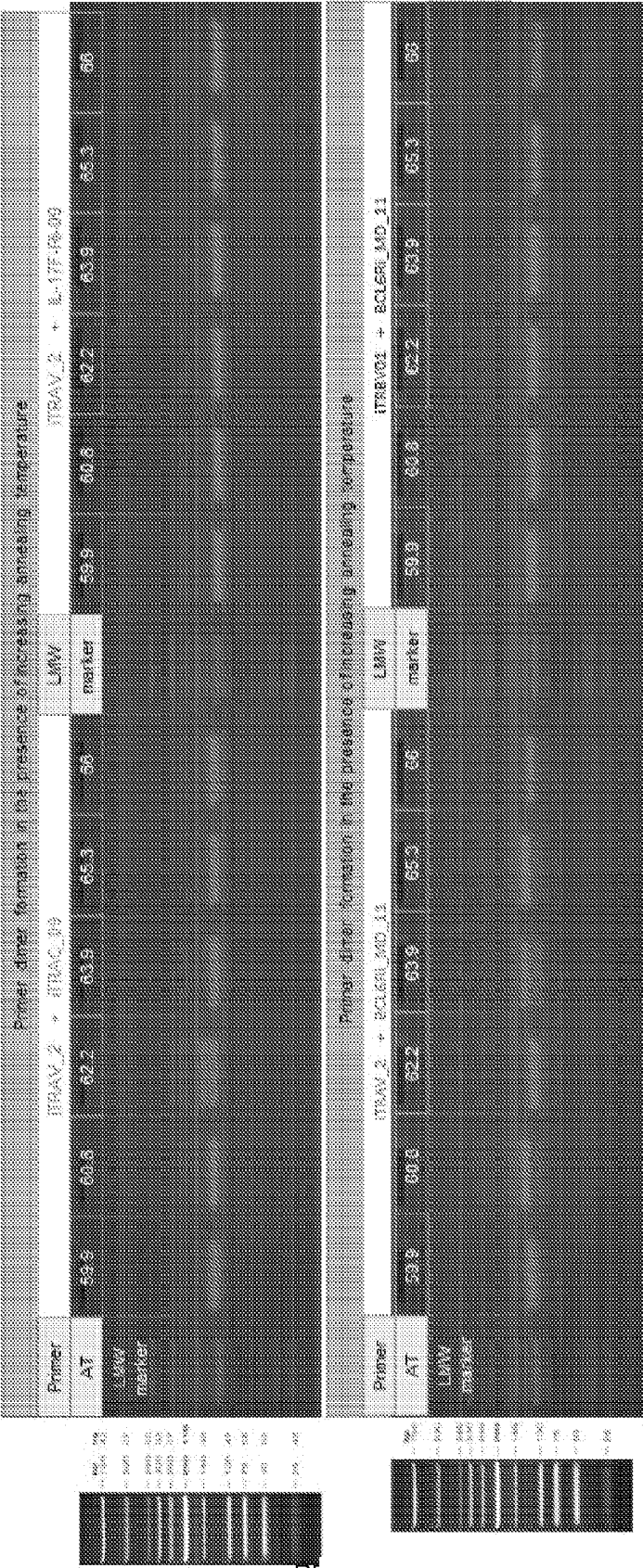


FIG. 4A

Top 3	Full Seq Read Primer Partial Read Seq	ITRAC AGTCTCTACCTGGTACAGC CGGTATAGTCTCTCAGCTGGTACACCGAGGTACGGTGGCCGTTT GCAGGTACGGTGGCCGTTT	ITRAC Full Seq Read Primer
Top 6	Full Seq Read Primer Partial Read Seq	IL-17F R1-00 ATGATAGGATGACAGGGGTGACGACG ATGATAGGATGACAGGGGTGACGACG GCAGGTACGGTGGCCGTTT	ITRAC Full Seq Read Primer
Top 2	Full Seq Read Primer Partial Read Seq	BCL2L1 MD_11 TACGTAGAGAGCGCAGGACGTGCACCTT GCAGGTACGGTGGCCGTTT	ITRAC Full Seq Read Primer
Top 2	Full Seq Read Primer Partial Read Seq	BCL2L1 MD_11 TACGTAGAGAGCGCAGGACGTGCACCTT TACGTAGAGAGCGCAGGACGTGCACCTT GCAGGTACGGTGGCCGTTT	ITRAC Full Seq Read Primer
Top 4	IL-17F R1-00 ATGATAGGATGACAGGGGTGACGACG ATGATAGGATGACAGGGGTGACGACG GCAGGTACGGTGGCCGTTT	Top 1 FOXO3 R1-00 ATGATATTAATCTTGGAGTTCAGTGGT ATGATATTAATCTTGGAGTTCAGTGGT GCAGGTACGGTGGCCGTTT	ITRAC Full Seq Read Primer
Top 3	BCL2L1 MD_11 TACGTAGAGAGCGCAGGACGTGCACCTT TACGTAGAGAGCGCAGGACGTGCACCTT GCAGGTACGGTGGCCGTTT	Top 7 CTLA-4 R1-00 ATGATATTAATCTTGGAGTTCAGTGGT ATGATATTAATCTTGGAGTTCAGTGGT GCAGGTACGGTGGCCGTTT	ITRAC Full Seq Read Primer

FIG. 4B



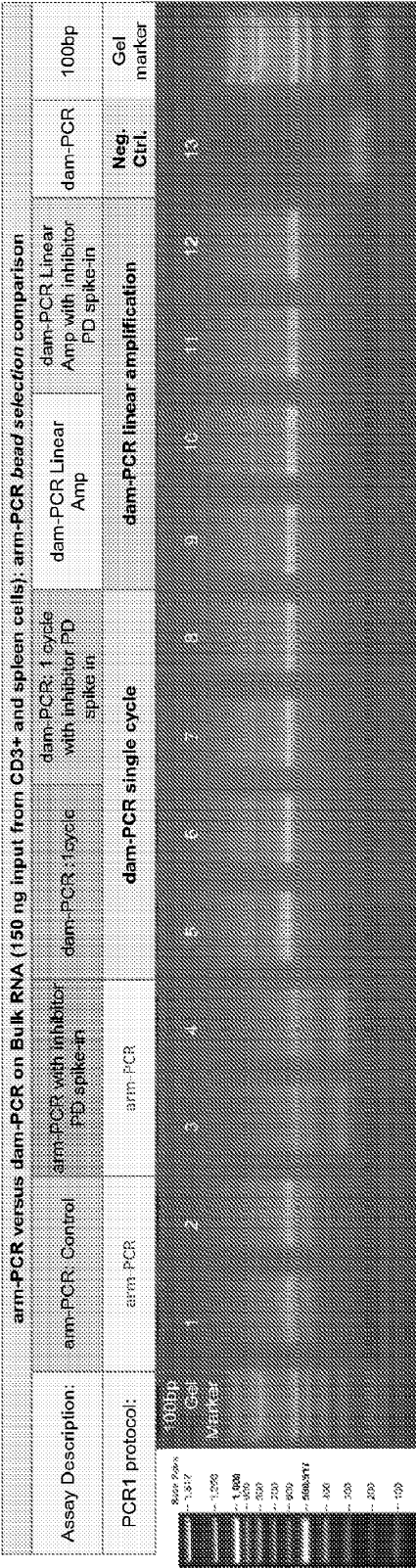


FIG. 7A

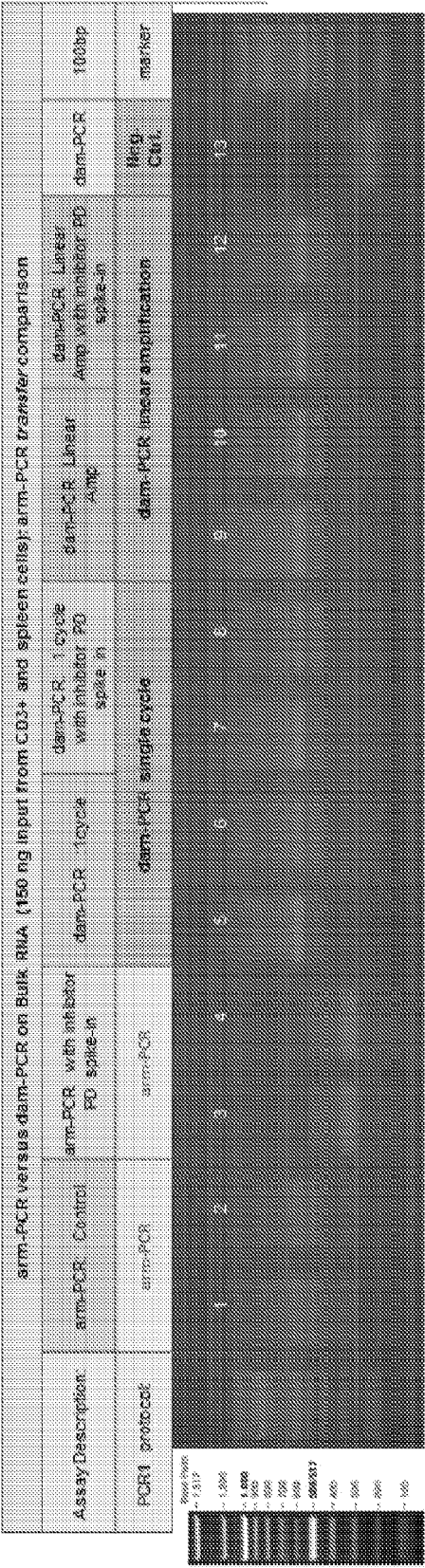


FIG. 7B

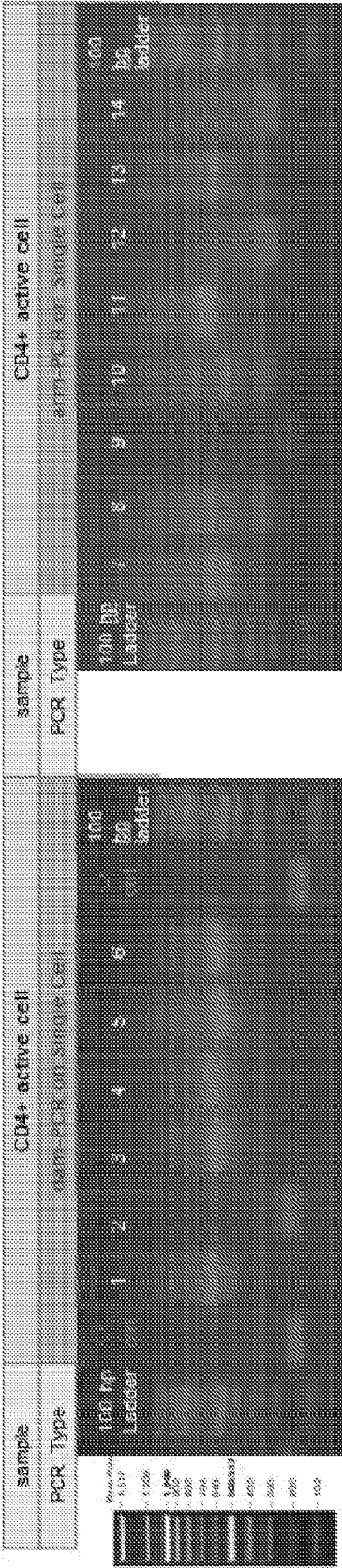


FIG. 8

[illegible]

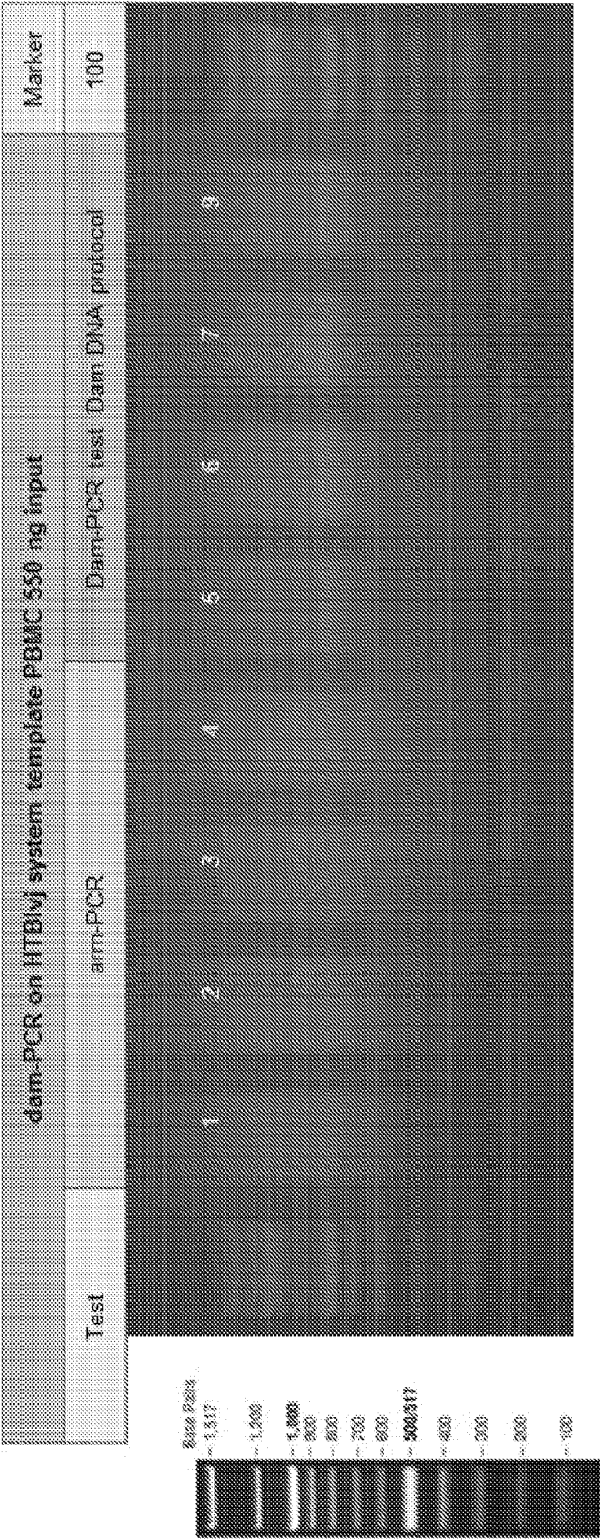


FIG. 10A

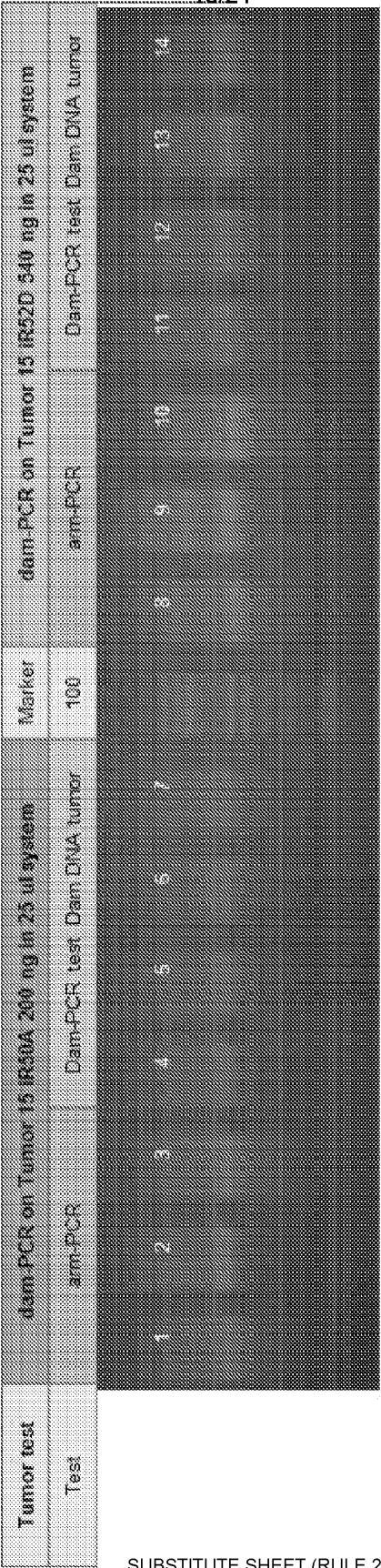


FIG. 10B

Larger Gene (gDNA) Targeted for Coverage with a Traditional Multiplex PCR Strategy

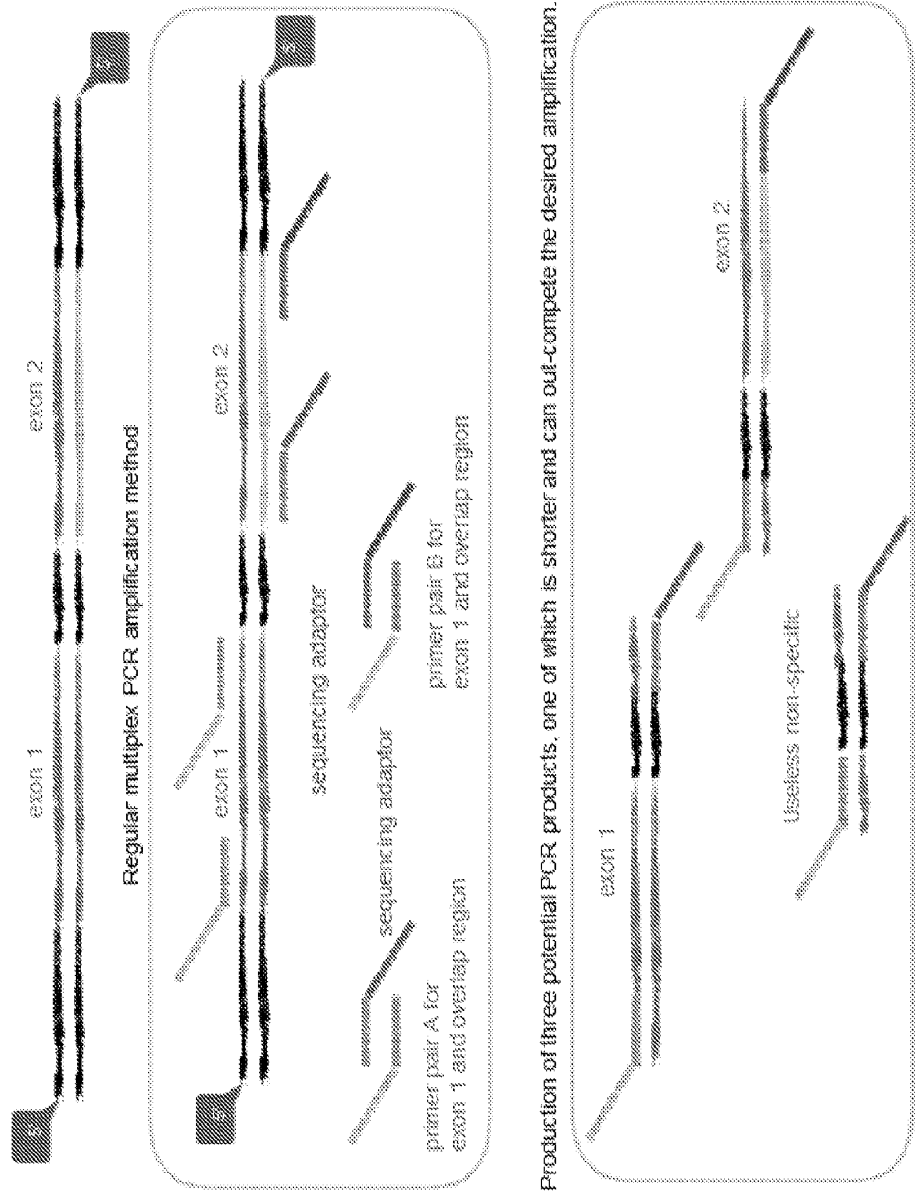


FIG. 11

Larger Gene (gDNA) Targeted for Coverage with dam-PCR

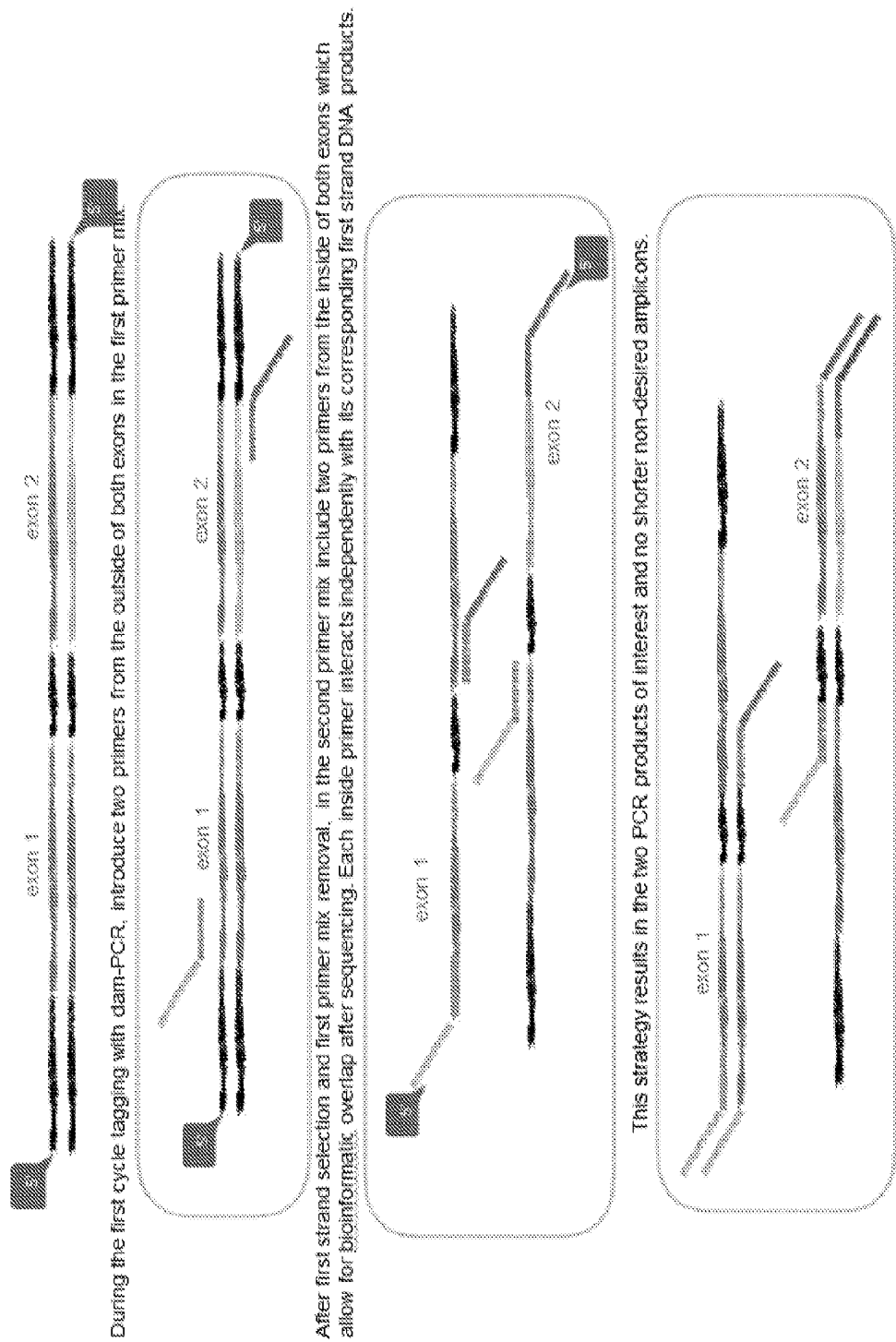


FIG. 12

dam-PCR gDNA amplification test on HLA overlap exon

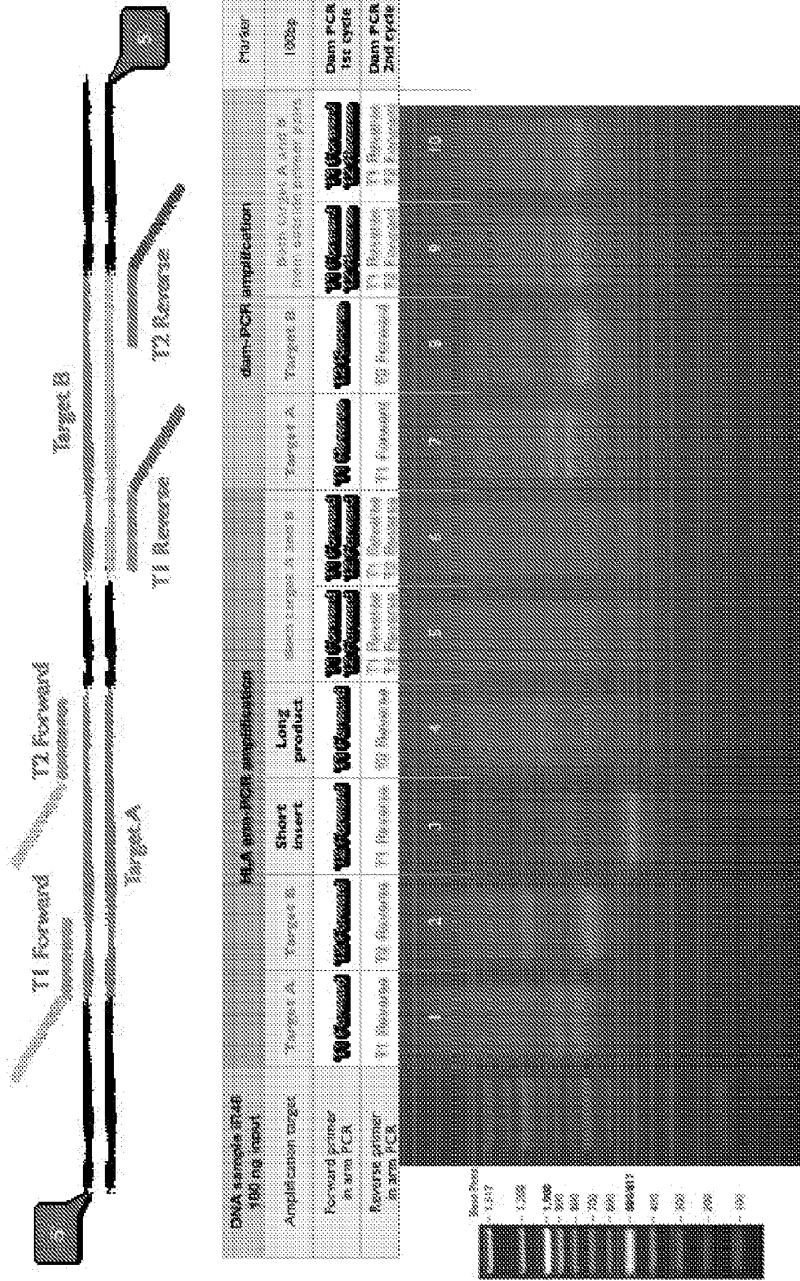


FIG. 13